

INFLUENCE OF AIR ON THE PRESERVATION AND AEROBIC SPOILAGE OF SILAGES

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ABSTRACT. *The effect of a surface open to air on the ensiling process was investigated in 15 × 60 cm PVC silos in four trials, three with alfalfa (30, 41, and 49% dry matter, DM) and one with whole-plant corn (35% DM). Silos were sampled 1, 2, 5, 7, 14, and 28 d post-filling at 5, 20, 35, and 50 cm from the open surface. At 5 cm, little fermentation occurred as oxygen contents were at 10% or higher, and spoilage microorganisms rapidly developed causing 37 to 55% DM loss over 28 d. At 20 cm and below, oxygen levels were initially at 1% or lower. This permitted lactic acid bacterial fermentations during the first week which appeared typical of fermentations under anaerobic conditions. However, after one week, the levels of fermentation products at the 20-cm depth began to differ from those at 35 and 50 cm. In general at the 20-cm depth, lactic acid content decreased, and acetic acid content either increased or decreased relative to concentrations at lower depths. At 28 d, noticeable spoilage was beginning to occur at the 20-cm depth, as evidenced by increasing pH and elevated populations of one or more groups of spoilage microorganisms. At the end of the trials, spoilage microorganisms were also elevated at the 35- and 50-cm depths relative to those normally expected in silages made under strictly anaerobic conditions.*

Keywords. *Silage, Oxygen, Bacteria, Yeast, Mold.*

In the U.S., crops are frequently ensiled without proper sealing. Concrete stave silos are rarely sealed with polyethylene after filling is completed. Consequently, a substantial spoiled layer may develop at the top of the silo prior to the start of emptying. In some instances, such as on small farms with one silo per crop, a farmer may remove forage from a silo before fermentation occurs. Bunker, trench, and drive-over pile silos are often not sealed because farmers may feel spoilage losses are not great enough to justify the time and cost of putting on polyethylene.

The consequences on long-term dry matter (DM) losses from failing to seal a silo are substantial. McLaughlin et al. (1978) observed DM losses in an uncovered bunker silo of 60 and 22% for the 0- to 25-cm and 25- to 50-cm depths, respectively. Similarly, Dickerson et al. (1990) surveyed covered and uncovered bunker silos on farms and found losses in the top 50 cm to be 27 and 41%, respectively. Bolsen et al. (1993) looked at losses in pilot- and small-scale bunker silos with various sealing treatments. In the uncovered pilot silos, there was a consistent progression of rapid spoilage in the upper 33 cm followed by increased spoilage at lower depths. At 84 or 90 d post-filling, there were significantly higher DM losses at the 67 to 100 cm depth from the open surface of the uncovered silos relative to those at the same depth in the covered silos in two of

three experiments. The authors measured fermentation products and pH at various depths from the surface. Even though substantial spoilage occurred in the top layer (0 to 33 cm) in all experiments, there was evidence of fermentation, particularly early in ensiling (7 or 14 d post-filling). These results suggest that normal fermentations may occur near the open surface.

The objective of this study was to more closely investigate the course of fermentation and the development of microorganisms in the top 50 cm of pilot-scale silos in both alfalfa and whole-plant corn silage. The goal was to determine how far below an open surface normal fermentation occurs and how deep must one go to find a relatively stable silage.

MATERIALS AND METHODS

Four similar trials were conducted in 1996, three with alfalfa and a fourth with whole-plant corn (table 1). The crops were harvested with typical forage harvesting equipment and packed by hand into 15-cm-diameter × 60-cm-long PVC pipe silos. The amounts ensiled and the densities achieved varied across the four trials (table 1), primarily because of variation in dry matter content. Twelve silos were filled in each trial. The bottom of each silo was sealed with a rubber cap and the top left open to air. Each

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Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by USDA, Agricultural Research Service.

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Table 1. Ensiling details for all four trials

Trial	Date	Dry Matter Content (%)	Wet Amount Ensiled (g)	Wet Density (kg/m ³)	Dry Density (kg/m ³)
1. 1st cut alfalfa	25 June	29.8	7800	736	219
2. 2nd cut alfalfa	16 July	49.0	6600	622	305
3. 3rd cut alfalfa	28 August	41.3	6600	622	257
4. Whole-plant corn	2 October	34.6	6500	613	212

silos was wrapped with approximately 9 cm of fiberglass insulation, and thermocouples were placed at 5, 20, and 35 cm from the open surface. Average hourly temperatures at each location were recorded on a Campbell 21X datalogger. All silos were stored inside at room temperatures (21–23°C).

Two silos were destructively sampled after 1, 2, 5 (or 3), 7, 14, and 28 d post-filling. Before sampling, 0.5-mL gas samples were collected via syringe through septa in the silo wall at 5, 20, 35, and 50 cm (in that order) from the open surface and immediately analyzed on a gas chromatograph for oxygen and carbon dioxide contents (Muck and Pitt, 1994). Then silage was carefully removed from each silo, and samples were taken at 5, 20, 35, and 50 cm from the open surface for analysis of DM content, pH, and microbial and chemical composition.

Three pre-ensiled forage samples from each trial and all silage samples were analyzed for the same constituents. The DM content was determined by freeze drying duplicate samples. The freeze-dried samples were ground and analyzed for crude protein by a Leco FP-2000A nitrogen analyzer and ash at 550°C for 3 h. Another portion of wet sample was diluted 10:1 with autoclaved distilled water and blended for 30 s. The diluted sample was analyzed for acid-tolerant lactic acid bacteria (LAB) using Rogosa SL agar (Difco #0480), enterobacteria, acetic acid bacteria, bacillus spores, yeasts and molds using selective media (Muck et al., 1992). The remaining diluted sample was strained through cheesecloth and analyzed for pH, fermentation products (Muck and Dickerson, 1988), and soluble nonprotein nitrogen (extracted in 5% trichloroacetic acid and analyzed by Leco FP-2000A).

RESULTS

PRE-ENSEILED CROP CHARACTERISTICS

The chemical and microbial compositions of the forages in the four trials are shown in tables 2 and 3, respectively. The first trial was with alfalfa of advanced maturity as evidenced by the lower crude protein content compared to the second and third trials (table 2). In all four trials, acid-tolerant lactic acid bacterial populations were sufficiently high that a rapid initiation of fermentation, producing lactic acid, was anticipated. With the exception of the acetic acid bacteria, counts of the other groups were similar to or less than the population of acid-tolerant LAB. In third-cut alfalfa and whole-plant corn, acetic acid bacteria were the dominant microbial group of those measured.

Table 2. Chemical composition of the crops ensiled in all trials

Trial	DM* Content (%)	Ash (% DM)	Crude Protein (% DM)	Soluble NPN† (% crude protein)	pH
1. 1st cut alfalfa	29.8	8.7	18.7	29.8	6.26
2. 2nd cut alfalfa	49.0	9.8	20.1	19.4	6.09
3. 3rd cut alfalfa	41.3	11.4	25.1	24.0	6.27
4. Whole-plant corn	34.6	3.3	7.9	9.8	5.67
S.E.‡	0.60	0.24	0.30	0.95	0.039

* Dry matter.

† Nonprotein nitrogen.

‡ Standard error; n = 3 replicates.

Table 3. Microbial composition [\log_{10} (colony forming units/g crop)] of the crops ensiled in all trials

Trial	Lactic Acid Bacteria	Entero- bacteria	Acetic Acid Bacteria	Bacillus Spores	Yeasts	Molds
1. 1st cut alfalfa	6.94	3.16	6.54	4.69	3.99	4.01
2. 2nd cut alfalfa	5.78	5.04	4.88	4.72	3.12	3.65
3. 3rd cut alfalfa	4.42	4.08	5.03	—*	4.58	4.70
4. Whole-plant corn	5.89	< 2.00	7.00	4.46	5.10	4.20
S.E.†	0.31	0.25	0.24	0.24	0.42	0.30

* Error in analysis; vegetative cells apparently were not completely killed.

† Standard error, n = 3.

GAS COMPOSITION

The oxygen and carbon dioxide profiles were similar in all three alfalfa trials, and typical changes with time and distance from the open surface are shown in figure 1A and 2A. Oxygen concentrations at a given depth were lowest at day 1. With time, the oxygen content at 5 cm below the open surface increased from 10 to 12% v/v on day 1 to 17 to 19% on day 28. At 20 cm, oxygen content was approximately 1% at 1 d post-filling and increased to approximately 2% on day 28 in all three alfalfa trials. At 35 and 50 cm, oxygen contents were relatively constant from day 1 through 28 and generally below 1%, which was near the detection limit of the measurement system. Carbon dioxide contents were highest on either day 1 or 2, and concentrations increased with distance from the open surface (fig. 2A). At a given depth, carbon dioxide concentration declined with time

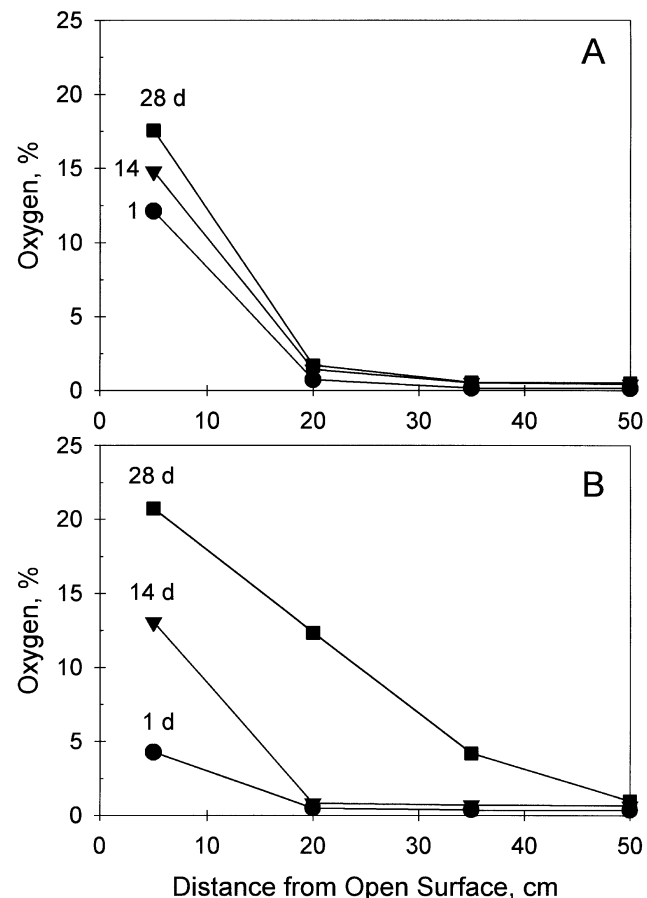


Figure 1—Oxygen profiles on different days post-filling in first cut alfalfa (A) and whole-plant corn (B) silages.

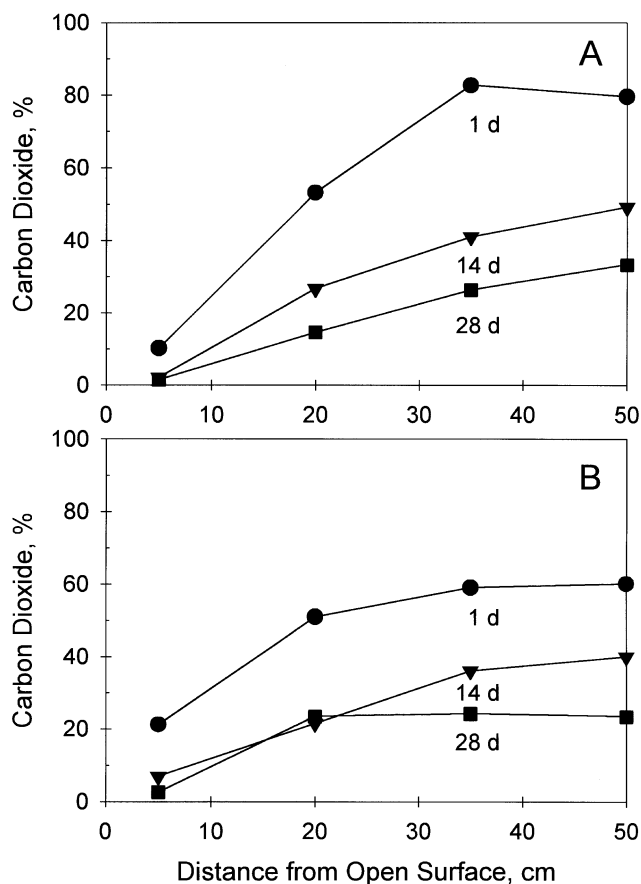


Figure 2—Carbon dioxide profiles on different days post-filling in first-cut alfalfa (A) and whole-plant corn (B) silages.

thereafter. The rate of decline was slowest in first-cut alfalfa (shown in fig. 2A) and fastest in the most porous silage (second-cut alfalfa; data not shown).

Gas composition in the corn silage followed a similar pattern to that in the alfalfa trials (figs. 1B, 2B). The major difference was the oxygen profile at 28 d. By this time, much higher levels of oxygen were present at the 20- and 35-cm depths than were observed in any of the alfalfa trials.

LACTIC ACID BACTERIA AND FERMENTATION

The development and changes in total acid-tolerant LAB were similar in each of the three alfalfa trials, and only the results from first cut alfalfa are shown (fig. 3A). At 1 d post-filling, LAB numbers were similar at all four depths and near or greater than 10^9 colony forming units (cfu)/g silage. At 5 cm, numbers were declining by day 7 and continued declining to a minimum of 1000 to 3000 cfu/g on day 14. Counts on day 28 were consistently higher than those on day 14 by two to tenfold. At the lower depths, LAB counts were relatively constant between days 1 and 28, with the exception of day 28 at the 20-cm depth where there was a trend toward a lower population than at 35 and 50 cm.

In corn silage, LAB counts were above 10^9 cfu/g at 1 d post-filling (fig. 3B). By day 7, counts were declining at all depths except at 5 cm. The LAB population continued to decrease with time (14 and 28 d), with the exception of the LAB population at the 20-cm depth on day 28.

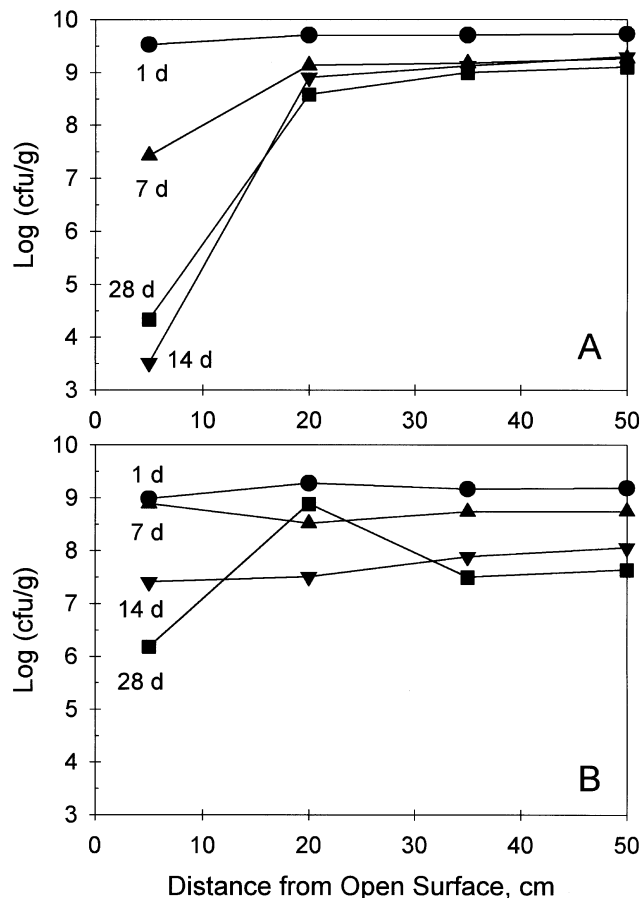


Figure 3—Lactic acid bacterial counts on different days post-filling and depths from the open surface in first-cut alfalfa (A) and whole-plant corn (B) silages.

Because of the rapid development of LAB, fermentation products (lactic and acetic acids, ethanol) were observed even at 5 cm early in the ensiling process in all four trials. Typical results are shown for lactic and acetic acids in two trials (figs. 4, 5). However, a pH decline was not consistently found at 5 cm in the first 2 d post-filling (fig. 6A). Lactic acid concentrations at 5 cm decreased after day 1 and were below detectable level by day 14. Trends in acetic acid and ethanol contents were less consistent, peaking on day 1 or 2 and then declining to low concentrations. These declining acid concentrations also coincided with increasing pH values with time at the 5-cm depth, both indicative of spoilage.

At 20 cm and lower from the open surface, lactic acid, acetic acid, and ethanol contents within a trial generally increased in a similar manner during the first week (figs. 4, 5), and pH values across these three depths were similar through the first two weeks (fig. 6) in all four trials. However by day 14, lactic acid concentration at 20 cm had dropped in three of four trials compared to the concentrations at 35 and 50 cm. By day 28, there was at least a trend at 20 cm for increased pH in all four trials and reduced acetic acid content in three of four trials relative to values at 35 and 50 cm (figs. 4, 5). Ethanol content remained relatively constant after the first week at 20 cm and below in the three alfalfa trials. In corn silage, ethanol content peaked on day 14 at 35 and 50 cm. At 20 cm, ethanol declined after the first week and was not detected in the 28-d silages.

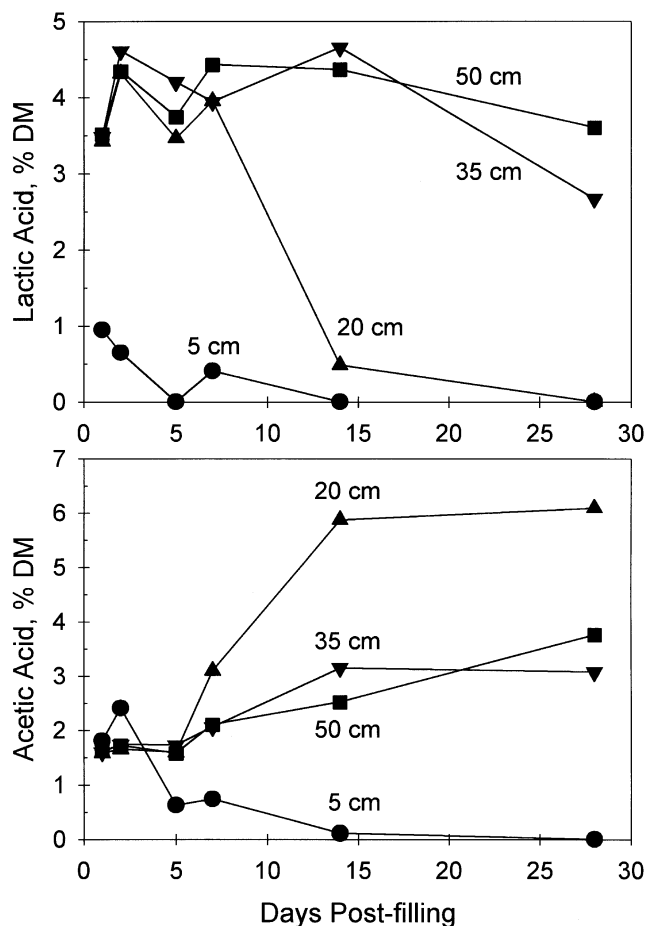


Figure 4—Lactic and acetic acid contents in first-cut alfalfa silage as affected by days post-filling and distance from the open surface.

ENTEROBACTERIA

The enterobacteria, anaerobic competitors of the LAB, were active early in the ensiling process. In first-cut alfalfa and corn, the peak numbers were observed after 1 d post-filling, but by day 7 numbers were declining except at 5 cm (fig. 7). In the later alfalfa trials, counts on day 1 were similar to those in the pre-ensiled forage (table 3). Counts increased on day 2 (data not shown) in these two trials and then began to decline. By day 14, counts of enterobacteria were below detectable level (100 cfu/g) at all depths and in all trials with the exception of the first trial (fig. 7A), the wettest alfalfa trial.

TEMPERATURE

Temperatures followed similar patterns in all four trials. Temperatures as a function of time and distance from the open surface are shown in figure 8 for two of the trials. Air temperature in the laboratory was relatively constant (21 to 23°C) over all four trials with the exception of several days in the middle of the corn silage trial (fig. 8B). The higher air temperatures in this period were caused by an air conditioning failure and had a noticeable effect on silage temperatures as well.

The highest temperatures in the silages developed at the 5-cm depth. The pattern of temperature at this depth was characterized by two peaks, the first within 24 to 36 h post-filling and the second at 3 to 5 d post-filling. After the

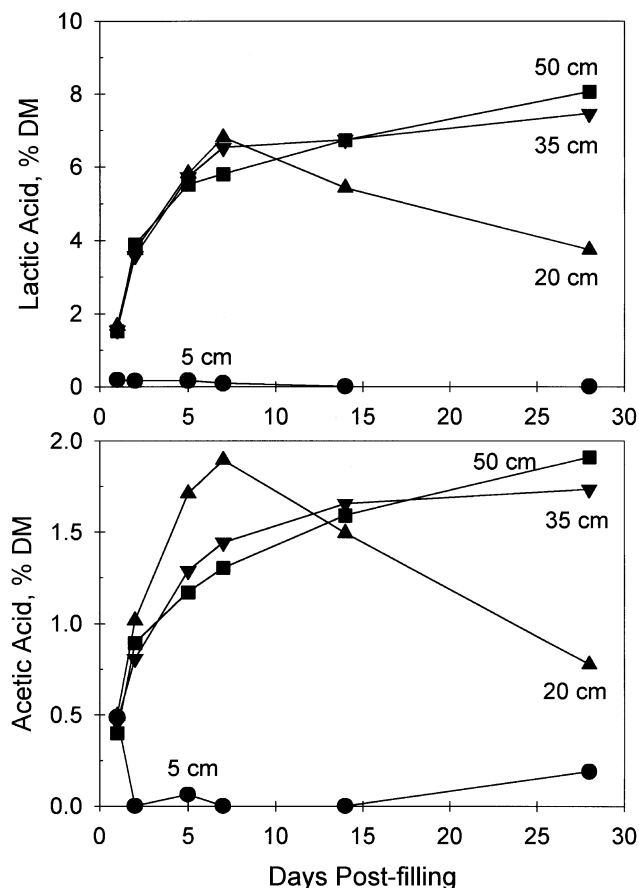


Figure 5—Lactic and acetic acid contents in whole-plant corn silage as affected by days post-filling and distance from the open surface.

second peak, temperatures declined gradually thereafter. At the 20-cm depth, temperatures in the first two weeks were intermediate of those in air and at 5 cm, suggesting heat transfer from the active heating zone around the 5 cm level because little oxygen was present at 20 cm (fig. 1) to support respiration. Late in the trials as oxygen content increased at 20 cm, temperatures at that depth eventually were slightly higher than those at 5 cm. At the 35-cm depth, temperatures more closely paralleled air temperature.

AEROBIC MICROORGANISMS

Variations in the populations of aerobic spoilage microorganisms (acetic acid bacteria, bacilli, yeasts, and molds) were distinctly different between alfalfa and corn, particularly for the fungi. Changes in yeasts and molds with time and distance from the open face are shown in figure 9 and 10, respectively, for the first cut alfalfa and corn silages. In alfalfa, the only increase in yeast counts occurred at the 5-cm depth during the first day post-filling. At other depths during the first 24 h, yeast counts either stayed the same or declined relative to the initial population. Thereafter, average yeast counts decreased to or below the detectable level (100 cfu/g) at all depths by 14 d. By day 28, no yeasts were detected on any of the alfalfa samples. In contrast, yeast counts in corn silage at the 5-cm depth immediately rose to 10^8 cfu/g during the first day post-filling and remained above that level

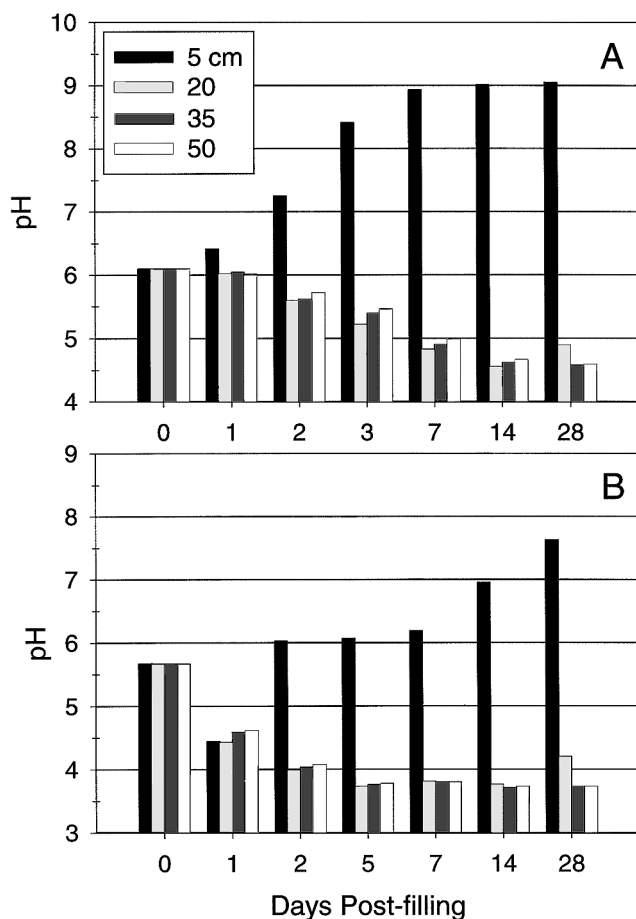


Figure 6—Silage pH at different days post-filling and distances from the open surface in second-cut alfalfa (A) and whole-plant corn (B) silages.

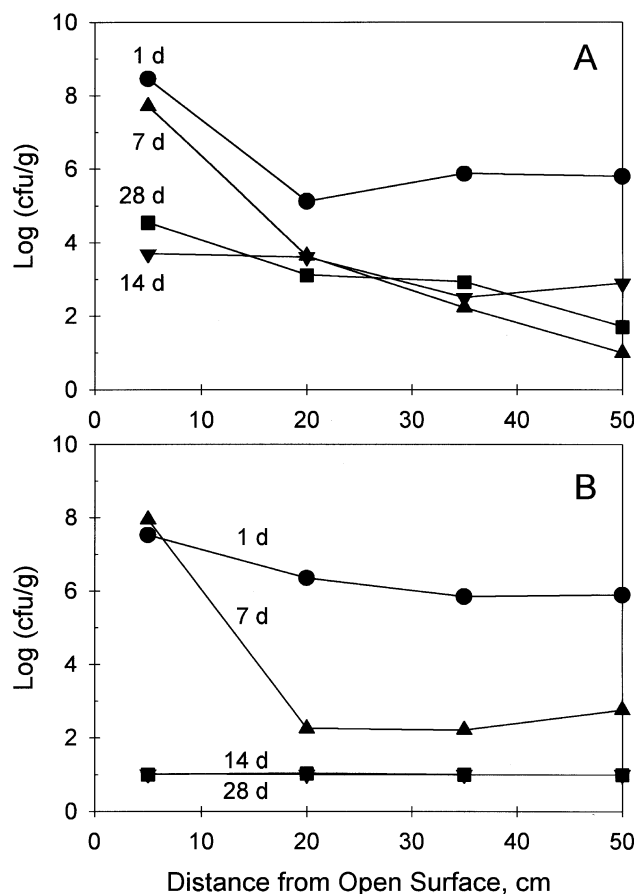


Figure 7—Enterobacterial counts in first-cut alfalfa (A) and whole-plant corn (B) silages as affected by days post-filling and distance from the open surface. Detectable level is 2 log(cfu/g); 1 log(cfu/g) represents having all replicates below detectable level.

Table 4. Chemical composition of the 28-d silages in all trials

Trial	Depth (cm)	DM* Content (%)	Ash (% DM)	Crude Protein (% DM)	Soluble NPN† (% crude protein)	Ethanol (% DM)
1. 1st cut alfalfa	5	29.8	16.49	19.5	30.4	ND‡
	20	26.2	9.79	19.8	47.7	0.20
	35	27.1	9.69	19.9	49.0	0.31
	50	27.4	9.70	19.6	49.6	0.27
2. 2nd cut alfalfa	5	55.2	16.12	21.7	32.1	ND
	20	48.5	9.95	21.3	37.9	0.13
	35	50.3	9.98	21.0	41.1	0.17
	50	51.3	9.91	21.6	34.6	0.16
3. 3rd cut alfalfa	5	52.6	17.97	22.7	29.5	ND
	20	40.1	11.69	25.8	44.4	0.15
	35	41.7	12.22	24.8	40.5	0.17
	50	42.4	12.05	24.6	52.0	0.22
4. Whole-plant corn	5	43.1	7.30	14.5	38.7	ND
	20	29.9	3.65	8.4	75.4	ND
	35	32.9	3.19	8.2	80.0	0.60
	50	33.7	3.46	8.6	72.0	0.82
S.E.§		2.7	0.37	0.5	4.8	0.13

* Dry matter.

† Nonprotein nitrogen.

‡ Not detected.

§ Standard error, n = 2.

throughout the 28 d. At 20 cm, counts rose to 10^6 cfu/g and remained relatively constant. At 35 and 50 cm, yeasts counts were relatively unchanged during the first 24 h post-filling. Thereafter, counts at the two lowest depths increased during the first week, declined in the second and then rose to the highest levels (10^6 cfu/g) on day 28.

Mold counts in alfalfa silage decreased or remained the same during the first day post-filling, and counts were unaffected by depth (fig. 10A). With the exception of the 5-cm depth in first cut alfalfa, mold counts at all depths and in all three alfalfa trials generally decreased during the first two weeks. However, on day 28, mold counts had risen to approximately 10 000 cfu/g at the 20-cm depth in all three alfalfa trials, and counts had increased at one or both of the 35- and 50-cm depths. In contrast, mold counts in corn silage at the 5-cm depth immediately increased during the first 24 h post-filling to over 10^6 cfu/g, continued increasing over the first two weeks, and remained high at day 28 (fig. 10B). At 20 cm, mold counts declined slowly over the first two weeks and then rose to over 10^5 cfu/g by day 28. At 35 and 50 cm, a similar pattern to the 20-cm depth occurred through day 14. On day 28, both replicate silos had higher levels of molds at the 50-cm depth than at 35 cm.

No consistent pattern was observed in the changes in acetic acid bacterial counts with time and depth among the four trials (fig. 11). In both first-cut alfalfa silage (the wettest alfalfa trial) and corn silage (fig. 11A, D), counts

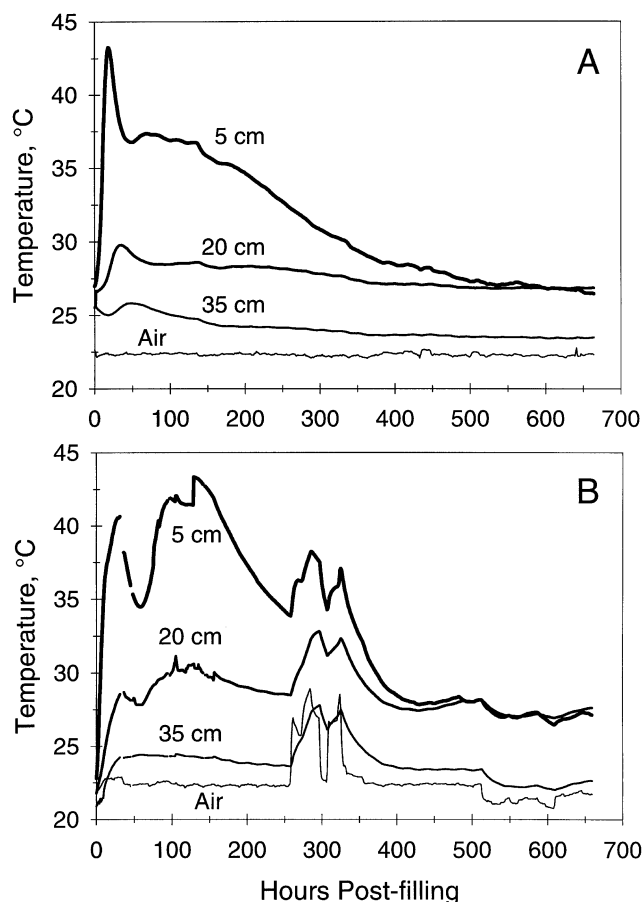


Figure 8—Temperature in third-cut alfalfa (A) and whole-plant corn (B) silages as affected by days post-filling and distance from the open surface.

increased to more than 10^8 cfu/g over the first 24 h post-filling and remained high during the first week. Thereafter, counts dropped slowly in the corn silage over the next week and continued to decline during the final two weeks except at the 20-cm depth. In the first cut alfalfa silage, the numbers dropped to below detectable level during the second week, and then numbers increased at the 20 through 50 cm depths during the final two weeks to between 1000 and 10 000 cfu/g. In second-cut alfalfa silage (fig. 11B), numbers at the 5-cm depth rose during the first 24 h and then declined sharply over the first week to below detectable level, remaining low for the duration of the trial. At 20, 35, and 50 cm, counts were relatively constant during the first week, increased to approximately 10^7 cfu/g during the second week and then dropped to 10^3 to 10^5 cfu/g at the end of four weeks. In third-cut alfalfa silage (fig. 11C), counts rose to almost 10^8 cfu/g at the 5-cm depth during the first 24 h. However, by the end of the first week, counts had dropped below 10^5 cfu/g, and on day 14, counts were below detectable level. At the other depths, counts increased to above 10^7 cfu/g by the end of the first week and remained above that level for the duration of the trial.

Bacillus spore counts had a similar pattern in all three alfalfa trials, and variation with time and depth for only the first trial is shown (fig. 12A). Counts increased rapidly at the 5-cm depth during the first week post-filling to greater

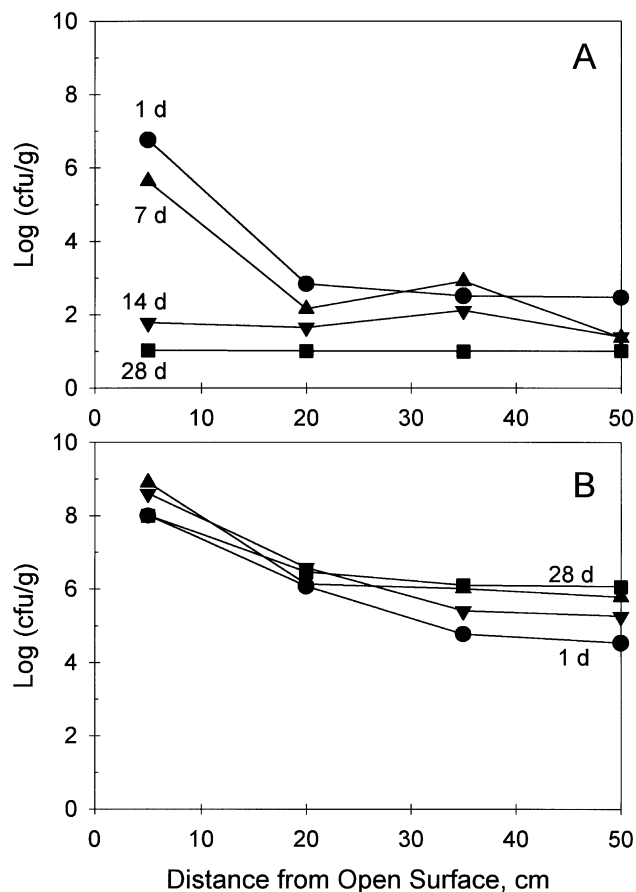


Figure 9—Yeast counts in first-cut alfalfa (A) and whole-plant corn (B) silages as affected by days post-filling and distance from the open surface. Detectable level is 2 log(cfu/g); 1 log(cfu/g) represents having all replicates below detectable level.

than 10^9 cfu/g and remained high for the duration of each trial. At the lower depths, spore counts generally peaked during the first two weeks, the highest count being at 50 cm in two of the three trials. At the end of four weeks, spore counts were consistently at 3×10^6 cfu/g at the 20-cm depth and generally between 10^5 and 10^6 cfu/g at 35 and 50 cm. In corn silage (fig. 12B), bacillus spore counts at the 5-cm depth were similar to those at 5 cm in the alfalfa trials. However, the pattern at the lower depths was different. Spore counts increased during the first 24 h post-filling and then declined over the first week to the lowest counts observed. After day 7, spore counts increased slowly with time.

ASH CONTENT

Ash content is a good indicator of DM loss during ensiling because it is not lost except in the case of silage effluent. In all trials, ash content at the 5-cm depth increased rapidly during the first week. Over the final three weeks, ash levels at 5 cm continued to increase with time but at a declining rate. Final ash contents (table 4) at this depth were between 58 and 121% higher than those in the forages at filling, the highest in corn silage and the lowest in third-cut alfalfa silage. Increases in ash content over four weeks at the lower three depths were generally small (12% and less), and final ash contents at 20 cm were not consistently higher than those at 35 or 50 cm (table 4).

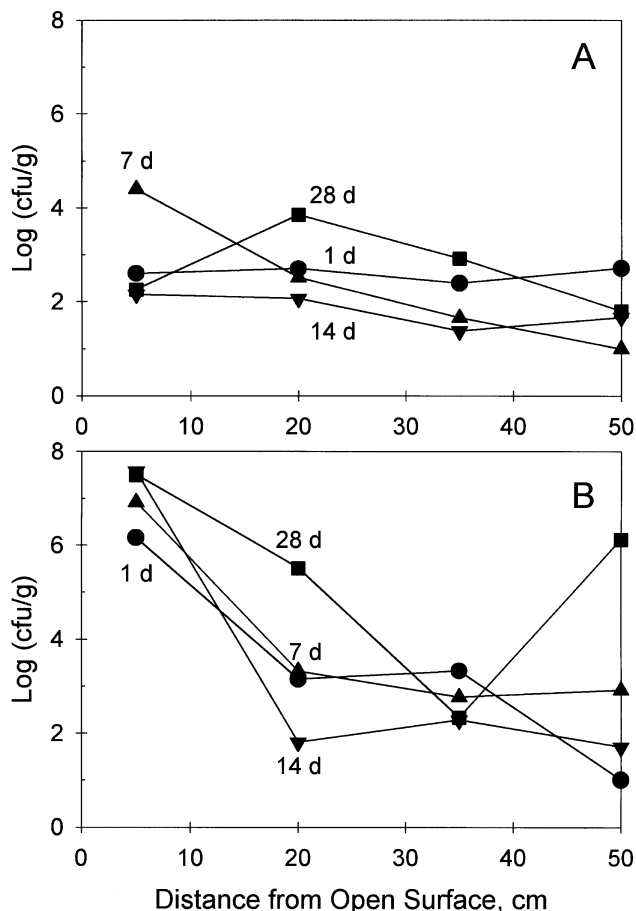


Figure 10—Mold counts in first-cut alfalfa (A) and whole-plant corn (B) silages as affected by days post-filling and distance from the open surface. Detectable level is 2 log(cfu/g); 1 log(cfu/g) represents having all replicates below detectable level.

SOLUBLE NONPROTEIN NITROGEN

Increases in soluble NPN with time at the 20-, 35-, and 50-cm depths were generally similar, and the majority of NPN formation occurred during the first week. Soluble NPN in the 28 d silages are shown in table 4. At the 5-cm depth, less soluble NPN was produced than at the three lower depths. In general, soluble NPN in the 28-d silages at 20, 35, and 50 cm were not significantly different within a trial.

DISCUSSION

Some patterns were consistent over all four trials. First, the rate of oxygen utilization by plant and microbial respiration was high enough to essentially prevent oxygen from diffusing much beyond 20 cm at least during the first two weeks of the ensiling process. Muck and Pitt (1994) in studying aerobic deterioration at the silo face found a similar oxygen profile once spoilage microorganisms near the open surface reached 10^8 cfu/g and silage temperatures began to increase relative to ambient temperatures.

Second, this utilization of oxygen near the open surface in the present study permitted very similar fermentation patterns (i.e., lactic and acetic acid contents) at the 20-, 35-, and 50-cm depths over the first week post-filling in all four trials. These fermentations resulted in pHs at those depths

that are typical of well fermented alfalfa (4.5 to 5.0) and corn silages (< 4.0).

Third, as each trial progressed, the rapidly respirable portion of the ensiled forage at the 5-cm depth began to be exhausted. This was evidenced primarily by declining temperatures at 5 cm after one week post-filling and a slowing in the rate of increase in ash content at this level.

Fourth, as rapidly respirable substrate near the open surface was exhausted, oxygen penetrated further into the silo. This, in turn, led to changes in fermentation products, pH and microbial populations at the 20-cm depth. After one week, lactic to acetic acid ratio at 20 cm tended to decrease relative to those at 35 and 50 cm through a conversion of lactic acid to acetic acid, an increased rate of acetic acid production relative to lactic acid, or a loss of both lactic and acetic acids. The former two pathways were likely due to shifts in substrates and products from lactic acid bacteria growing in the presence of oxygen (Condon, 1987). The latter was most likely due to aerobic microbial respiration. By day 28, there was either a trend toward or significantly higher pH and lower levels of fermentation products at 20 cm than at 35 and 50 cm with the exception of acetic acid in first cut alfalfa silage.

Fifth, there was evidence of more active spoilage at the 20-cm depth by the end of all trials. By 28 d, temperatures at 20 cm were consistently exceeding those at 5 cm, and mold counts were increasing at 20 cm in all four trials. Also, acetic acid bacteria counts were increasing at the 20-cm depth in corn silage.

Sixth, enterobacteria are normally expected to be suppressed in silages by low pH (McDonald et al., 1991). In three of the four trials, enterobacterial populations were below detectable levels after 28 d even in the spoiled silage at the 5-cm depth where limited fermentation occurred and where pH dropped below 6.0 in only one trial. The exception was the wettest alfalfa silage (30% DM) where enterobacterial counts were at approximately 10^4 cfu/g on day 28.

Seventh, reduced proteolysis occurred in all four trials at the 5-cm depth, where oxygen was always present. These results coincide with experiments where controlled oxygen concentrations were observed to reduce proteolysis in alfalfa silages (Makoni et al., 1997).

Finally, while "normal" fermentations occurred at the 35- and 50-cm depths, these silages generally contained high or increasing populations of one or more groups of spoilage microorganisms. Mold and yeast populations were increasing in corn silage. Mold counts were generally increasing in all three alfalfa trials at those depths, and acetic acid bacterial counts were high in one trial and increasing in another. This is not typical of completely anaerobic conditions where mold and acetic acid bacterial counts drop below detectable levels and remain low (e.g., Muck et al., 1992). The results in the present study suggest that active spoilage at 35 and 50 cm could have been initiated by molds if the trials had been performed for a longer duration. This would be different than the normally observed pattern of spoilage in silages during feedout, which is initiation by yeasts or acetic acid bacteria, followed by bacilli once pH has risen, and finally followed by mold development at a much later time (Spoelstra et al., 1988; Woolford, 1990; Muck and Pitt, 1994).

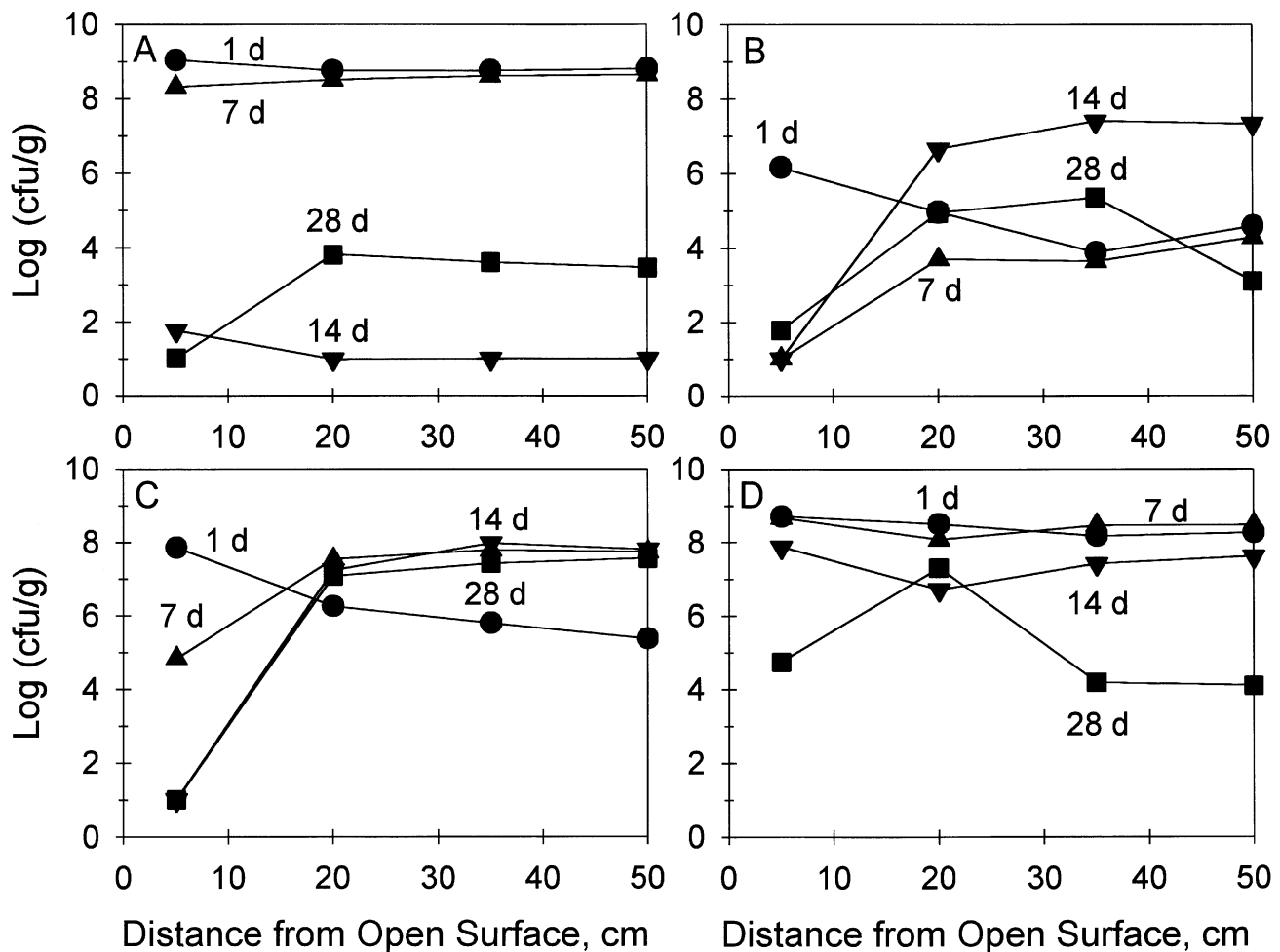


Figure 11—Acetic acid bacterial counts as affected by days post-filling and distance from the open surface in first-cut alfalfa (A), second-cut alfalfa (B), third-cut alfalfa (C), and whole-plant corn (D) silages. Detectable level is 2 log(cfu/g); 1 log(cfu/g) represents having all replicates below detectable level.

Deterioration in corn in the essentially unfermented crop at 5 cm followed a pattern that was similar to that previously observed in corn silage (Spoelstra et al., 1988; Muck et al., 1992; Muck and Pitt, 1994). Yeasts and acetic acid bacteria appeared to be very active initially with populations above 10^8 cfu/g at 24 h post-filling. By day 5, bacillus spore counts were above 10^8 cfu/g and molds above 10^6 cfu/g, suggesting their importance later in the spoilage process.

In the alfalfa trials, the acetic acid bacteria were the first measured aerobic group to develop at the 5-cm depth. They were followed within a day or two by bacilli. Yeasts and molds were not a factor in spoilage at this depth except for days 2 and 3 in the first trial with the 30% DM alfalfa where their brief presence at elevated levels occurred between the development of the acetic acid bacteria and bacilli. The general suppression of yeast and mold growth in alfalfa silage has been observed (O'Kiely and Muck, 1992). In the present study, this suppression appears to have occurred even in a zone where little fermentation was observed.

Dry matter losses can be calculated from the increase in ash content. At the 5-cm depth, ash content rose between 58 and 121%, depending on the trial. This represents a DM loss of 37 to 55% after 28 d of ensiling. These losses are typical of top spoilage losses measured in both farm-scale

and pilot-scale silos (McLaughlin et al., 1978; Dickerson et al., 1990; Bolsen et al., 1993) as was discussed earlier.

An area of concern in these trials is the effect of the end cap on the process because the silos were only 60 cm long. Certainly if appreciable oxygen concentrations were present at the lowest depth, then it could be argued that the end cap may have artificially kept the oxygen content high at that depth. However from day 1 through day 28, oxygen content at 50 cm in all trials was at the detection limit of our system ($< 1\%$) and only in corn might there have been oxygen present above trace levels on day 28 (fig. 1B). So it does not appear that the end cap had an appreciable effect on oxygen concentration in our trials. This would not necessarily be true if the trials had been run for longer durations. Taller silos may have produced differences in carbon dioxide and nitrogen contents, but various ratios of these gases have been shown to have little effect on microbial development during the ensiling of whole-plant corn with the exception of yeasts (Muck et al., 1992).

Overall, these results confirm what was suggested by the data of Bolsen et al. (1993), namely a normal lactic acid bacterial fermentation can occur in an ensiled crop very close (20 cm) to a surface exposed to air. Obtaining a normal fermentation does not mean that the silage in that zone is protected from spoilage in the presence of oxygen.

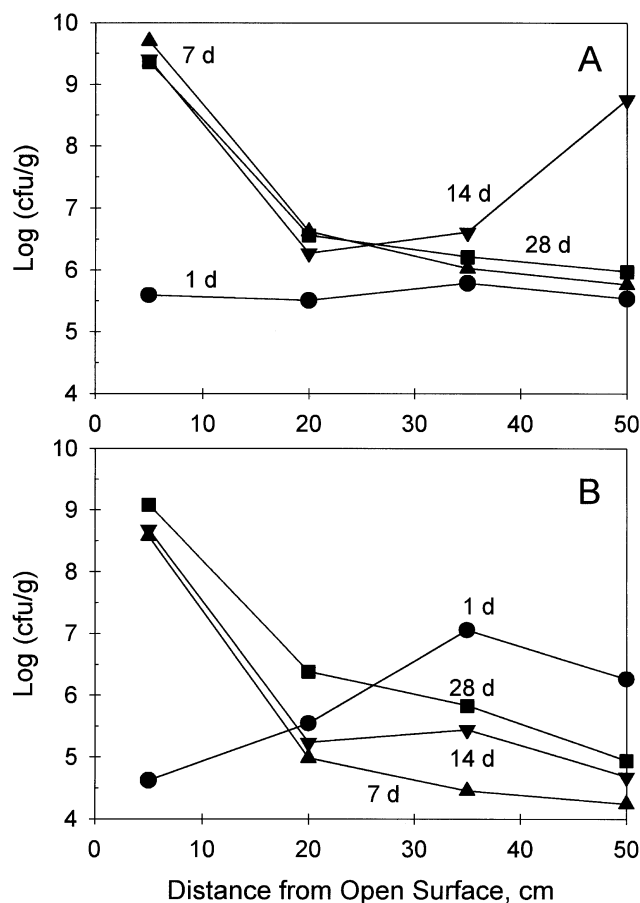


Figure 12—*Bacillus* spore counts in first-cut alfalfa (A) and whole-plant corn (B) silages as affected by days post-filling and distance from the open surface.

In fact, spoilage microorganisms did not consistently decline even at our lowest depth (50 cm), leaving silage at this depth and above more susceptible to heating and spoilage than properly stored silage. It is not clear from these trials how deep one must go from an open surface to find silage of similar aerobic stability as silage made in a well-sealed silo.

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